

Non-Invasive Methods For Determining Lesion Depth From Vesicant Exposure

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ABSTRACT

Before sulfur mustard injuries can be effectively treated, assessment of lesion depth must occur. Accurate depth assessment is important, because it dictates how aggressive treatment needs to be to minimize or prevent cosmetic and functional deficits. Depth of injury is typically assessed by physical examination. Diagnosing very superficial and very deep lesions is relatively easy for the experienced burn surgeon. Lesions of intermediate depth, however, are often problematic in determining the need for grafting. This study was a preliminary evaluation of three non-invasive bioengineering methodologies, reflectance colorimetry (RC), laser Doppler perfusion imaging (LDPI) and indocyanine green fluorescence imaging (ICGFI), to determine their ability to accurately diagnose depth of sulfur mustard lesions in a weanling swine model. Six female animals (8-12 kg) were exposed to 400 μ l of neat sulfur mustard on 6 ventral sites for 2, 8, 30, or 60 minutes. This exposure regimen produced lesions of varying depth from superficial to deep dermal. Evaluations of lesion depth using the bioengineering techniques were conducted at 24, 48, and 72 hours postexposure. Following euthanasia at 72 hours postexposure, skin biopsies were taken from each site and processed for routine H & E histological evaluation to determine the true depth of the lesion. Preliminary results demonstrated that LDPI and ICGFI were useful tools to characterize skin perfusion and provided a good estimate of HD lesion depth. The RC data are still being analyzed; however, the initial interpretation suggests that the data does not provide useful information concerning lesion depth. The novel prototype ICGFI instrument used in this study offered several advantages over LDPI and ICGFI instruments currently available including real-time imaging of blood flow through lesions, low cost, small size, portability, and not requiring the patient to be re-positioned. LDPI and ICGFI accurately predicted the need for aggressive treatment (30-and 60-min HD lesions) and non-aggressive treatment (2- and 8-min HD lesions) for the lesions generated in this study. Additional experiments are required to determine the exposure time necessary to produce a graded series of partial thickness HD lesions. The data generated will allow for a full assessment of the potential LDPI and ICGFI hold for predicting the need for aggressive treatment following HD exposure.

Key Words: reflectance colorimetry, laser Doppler perfusion imaging, indocyanine green fluorescence imaging, sulfur mustard lesion depth, and wound healing strategies

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INTRODUCTION

Accurate wound depth assessment is important in dictating the degree of aggressive treatment to minimize or prevent cosmetic and functional deficits from a vesicant lesion. In chemical burns, depth of injury is typically assessed by physical examination. Surface appearance, the pinprick test to assess pain, the “blanch-capillary return test” to evaluate microcirculation, and surface temperature difference between burned and unburned skin are often utilized in diagnosis of depth.¹ Utilizing these methods, diagnosing very superficial and very deep burns is relatively easy for the experienced burn surgeon. Burns of intermediate depth are often problematic in determining the need for aggressive treatment including grafting.

Non-invasively examining cutaneous blood flow, using available bioinstrumentation, can greatly assist the physician in making depth of injury determinations. Laser Doppler perfusion imaging, indocyanine green fluorescence imaging, and reflectance colorimetry may prove to be very valuable tools in diagnosing depth of vesicant injuries.

Laser Doppler flowmetry and laser Doppler perfusion imaging (LDPI) have been used for prolonged, non-invasive monitoring of tissue viability and wound healing, and for the assessment of peripheral vascular disease, inflammation, ischemia, reperfusion, skin graft acceptance (take) and burn depth.²⁻¹⁷ LDPI has proven useful in delineating the areas of damage that need to be debrided, avoiding areas with sufficient blood flow. Brown *et al.*¹⁸ found that laser Doppler perfusion images of vesicant vapor burns on the backs of swine correlated well with histopathological findings (thrombosis and necrosis of sub epidermal capillaries) between 1 h and 7 days post-exposure, and suggested that clinical management decisions on how to treat early vesicant burns could be aided by LDPI. Chilcott *et al.*¹⁹ used several non-invasive bioengineering methods to monitor wound healing in a large white pig model for 7 days following exposure to sulfur mustard (SM) and Lewisite vapors. They concluded that while reflectance colorimetry and transepidermal water loss (TEWL) measurements could provide quantitative, non-invasive methods for determining efficacy of candidate treatment regimens, neither is comparable to the prognostic capabilities of LDPI. Graham *et al.*²⁰ found LDPI to be useful in examining blood flow in grafted and un-grafted sites following treatment of deep dermal/full-thickness liquid SM injuries in a weanling swine model. LDPI is currently rather time consuming if there are multiple sites to be evaluated and/or large images to be collected at high resolution. The length of scanning procedures could be decreased by increasing scanning speed (thus decreasing flux resolution), decreasing the size of the scan area, and/or decreasing the number of lines scanned within the scanning area (scan resolution). Improvements in the technology that will speed up LDPI without compromising image resolution are being developed.

Indocyanine green fluorescence imaging has also shown promise in determining burn depth based upon microcutaneous blood flow. It is a minimally invasive procedure that requires the placement of an intravenous line. Indocyanine green (ICG) is FDA approved for use in humans to determine cardiac output, hepatic function and liver blood flow, and for ophthalmic angiography. The fluorescence of intravenous ICG has been shown to estimate burn depth in small animals.²¹ In contrast to fluorescein fluorescence,²² ICG fluorescence is capable of distinguishing superficial and deep partial-thickness burns from full-thickness burns. The fluorescence intensity of ICG decreases exponentially with burn depth for burns of similar age.²³ ICG fluorescence was used to estimate burn depth in a porcine model using healing as an endpoint.²⁴ A prototype imaging system with a diagnostic algorithm, developed at the Wellman Laboratories of Photomedicine, Boston, MA, accurately diagnosed burns that healed within 21

days with minimal scarring from those that took longer to heal by secondary means. Measurements were made on burns created 2, 24, 48, and 72 hours prior to imaging. The algorithm was shown to be dependent on the age of the burn and independent on the location of the burn. This technology showed promise in plastic surgical applications^{25, 26} and accurate determination of thermal burn depth in humans.^{25, 27, 28} The advantages that this technology has over LDPI include greatly increased speed of image capture and ability to examine microcutaneous blood flow in real time. Multiple images over large areas can be captured in a relatively short period of time. Images are typically collected 5-10 minutes after ICG injection to allow uptake and distribution. The dye is then excited (e.g., 780 nm), and the resultant fluorescence emission (e.g., 825 nm) immediately captured and saved by a computer and analyzed for burn to normal skin fluorescence ratio. ICG binds strongly to plasma globulins, limiting both extravasations within burn-injured vascular epithelia and extravascular transport to areas nearby.²³ Large signals are thought to be the result of vasodilation and hyperemia, and smaller signals are thought to be attributable to vascular occlusion and edema.^{21, 23}

This study was a preliminary evaluation of three non-invasive bioengineering methodologies, RC, LDPI and ICGFI, to determine their ability to accurately diagnose depth of sulfur mustard lesions in a weanling swine model.

MATERIAL AND METHODS

Animal Model

Six female Yorkshire Cross pigs (weanlings), *Sus scrofa*, 8-12 kg, were used (Country View Farms). They were quarantined upon arrival for seven days and screened for evidence of disease before use. In conducting the research described in this report, the investigators adhered to the Guide for the Care and Use of Laboratory Animals by the Institute of Laboratory Animal Resources, National Research Council, in accordance with the stipulations mandated for an AAALAC accredited facility. Animals were supplied tap water *ad libitum* and fed approximately 300 g of Teklad Mini-Swine Breeder Sterilizable Diet (Harlan Teklad 7037, Harlan Teklad, Madison, WI) twice a day. Animals were housed individually in 4 x 6 ft pens with slatted aluminum floors. The animal holding room was maintained at $21^{\circ} \pm 2^{\circ}\text{C}$ with 50% \pm 10% relative humidity using at least 10 complete air changes per h of 100% conditioned fresh air. Animal rooms were maintained on a 12-h light/dark, full spectrum lighting cycle with no twilight.

Sulfur Mustard Exposure

Eighteen to 24 h before agent exposure, each animal was sedated by intramuscular (i.m.) injections of xylazine HCl (Rompun[®]; 1.0 mL @ 20 mg/mL) and a combination of tiletamine HCl and zolazepam HCl (Telazol[®]; weighed out in equal parts, reconstituted to 100 mg/mL, and 0.5 mL administered), and hair was removed from the ventral surface by chemical depilation using MAGIC Fragrant Shaving Powder (Carson Products Co., Savannah, GA).

On the morning of exposure, the pigs were anesthetized to a surgical plane of anesthesia with Rompun[®] (2.2 mg/kg) and Telazol[®] (6 mg/kg) i.m. Six exposure sites were set up on the ventral surface, 3 sites per side parallel to and approximately 2.5 cm lateral to the teat line and located between the axillary and inguinal areas. A plastic template was used for even spacing and consistent anatomical positioning of the sites among animals. In addition to the exposure

sites, 2 non-exposure control sites were located along the ventral midline, one located near the axillary area and the other near the inguinal area. Tape assemblies (5 cm x 5 cm) were prepared out of double-sided carpet tape and duct tape, with a 2.9-cm diameter hole punched through the center of each tape assembly. A circle of Whatman No. 2 glass microfiber filter paper (3.8 cm in diameter) was sandwiched between the carpet tape and the duct tape, centered over the 2.9 cm hole. A small bead of cyanoacrylate adhesive was placed along the periphery of the hole on the duct tape, and a rubber O-ring (31 mm inner diameter) glued onto the template. The templates were placed approximately 6 cm apart, center to center, onto the ventral surface, one centered in each of 6 exposure sites. The pig was placed in an agent hood in dorsal recumbency, supported by a stainless steel pig sling. A therapeutic heating pad (Gaymar Industries, Inc., Orchard Park, NY), with the circulating water temperature set at 41°C, was placed under the animal during the exposure period to minimize hypothermia. Using a pipette (Gilson, Pipetman P-1000), 400 μ l of undiluted HD was placed on each filter paper. A solid polytetrafluoroethylene cap liner (PTFE; 0.38 mm thick, sized for a 28 mm cap) was placed over the filter paper, followed by an appropriately sized rubber stopper (#7) to occlude the site and ensure complete contact of the wetted filter paper with the skin. The purpose of the O-ring was to keep the PTFE disk and rubber stopper in place.

An animal was exposed for 2, 8, 30, or 60 min. The 2- and 8-min sites were exposed one at a time using a 300-g weight to apply even pressure to the filter paper during the exposure period. The 30-min sites were exposed in a similar way except that all 30-min sites on an animal were done at the same time. The 60-min sites were exposed in a similar way except a rubber tile float was placed on top of the rubber stoppers to ensure even downward pressure. For the 60-min sites, VetrapTM bandaging tape (3M Animal Care Products, St. Paul, MN) was wrapped around the pig to secure the floats in place. At the end of the exposure period, all devices were removed, and the sites damped dry for 30 seconds with a double layer of absorbent Masslenn sports towel. The pigs were then placed into a holding cage under an adjacent hood. Once in the holding cage, water was provided *ad libitum*. Food was provided in the hood within a few hours of recovery from anesthesia. Buprenorphine (0.01 mg/kg i.m.) was administered immediately after exposure and early the following morning to alleviate any discomfort. Each pig was kept under the hood for approximately 24 h. The animals were not placed back into their pens until they tested negative for HD off-gassing using a MINICAMSTM (O.I. Analytical, CMS Field Products Group, Birmingham, AL).

Post-exposure Procedures

At each observation time post-exposure (24, 48, and 72 h) several procedures were conducted. If necessary, lesions were washed with water and dabbed dry. Reflectance colorimetry was conducted followed by Laser Doppler Perfusion Imaging and indocyanine green fluorescence imaging. Following the 72-h measurements, animals were euthanized and skin samples taken for histopathology examination.

Reflectance Colorimetry

The three-dimensional L^{*}a^{*}b^{*} color system recommended by the Commission Internationale de l'Eclairage in 1976 was utilized. Four replicate readings were taken from the center of each site. The median of the four individual measurements was calculated to correct for outliers. For each HD-exposed pig at each time point, the median L^{*}, a^{*} and b^{*} values of

similarly exposed sites were averaged, resulting in single L*, a* and b* values for each length of exposure. The net changes in lightness (L*), red/green balance (a*), and yellow/blue balance (b*) were calculated for each animal by subtracting the pre-exposure averages from the post-exposure averages. Net changes were also made in comparison with values from the mid-ventral control sites. Overall differences in color (E^*_{ab}) were calculated using the following formula:

$$\Delta E^*_{ab} = \sqrt{\Delta L^*^2 + \Delta a^*^2 + \Delta b^*^2}$$

Group mean, standard deviation (n-1) and standard error of the mean (SEM) were calculated for each length of exposure. The mean net changes in the L*, a* and b* chromaticity values, as well as overall color differences (E^*_{ab}), were plotted against exposure length.

Laser Doppler Perfusion Imaging (LDPI)

A moorLDITM (Moor Instruments, Inc., Wilmington, DE) was used for collecting images. The following operating parameters on the instrument were used: DC gain = 0, flux gain = 0, conc gain = 2, background threshold = 200, distance to subject = 20 cm, scan size = small, scan speed = 10 ms/pixel, DC image resolution = 256 x 256, and blood flux units (arbitrary units) set to “perfusion.” The scanner head was positioned perpendicular to the wound surface whenever possible. Blood flux levels at each of the pixel points within a defined region of interest (ROI) were measured and averaged, using built-in image analysis software. Each site was analyzed at each time point as follows. Five ROIs were measured within the lesion, each generating an average blood flux value. Those values were averaged, arriving at a single flux value (B) within the burn. Similarly, three ROIs were measured in normal perilesional skin nearest the injury (if available; on a contralateral site otherwise) and averaged, yielding a single flux value (N) for normal skin. A B/N ratio was then calculated. Obvious areas of hyperemia were avoided during the analyses.

Indocyanine Green fluorescence (ICG) Imaging

ICG fluorescence imaging was conducted using a lightweight, portable prototype instrument developed at the U.S. Army Medical Research Institute of Chemical Defense (Aberdeen Proving Ground, MD). The dye was excited with an emitting diode, and the resultant fluorescence emission was recorded with a charged coupled device (CCD) camera. Distance-to-subject was set at 24 inches, thereby standardizing the fluorescence (mJ/cm²) of the incident light. The optimal distance will need to be determined in future experiments. The images were captured and stored by a computer for analysis at a later time. Image-Pro[®] Plus 4.5 (Media Cybernetics, Silver Spring, MD) was used to perform image analysis. The brightness level of ICG fluorescence from the burns was determined from the images and was correlated with the depth of burn injury as determined by histology. These values were also compared with LDPI blood flux determinations. B/N ratios were calculated in a manner similar to that described above for LDPI, whenever nearby normal skin is available.

The dose of ICG (Becton Dickinson Microbiology Division, Cockeysville, MD) started at 0.2 mg/kg i.v. but switched to 0.8 mg/kg i.v. after the first animal. It was administered as a bolus through a central or peripheral venous line. The first two range finding pigs were given multiple boli. The total dose of dye injected was kept below 2 mg/kg, as recommended by the dye's

manufacturer. Uniformity of fluorescence detection was checked each day by recording the fluorescence of a 125-mm diameter disk (Whatman No. 2 filter paper) saturated with a 0.025 mg/ml ICG aqueous solution.

Each observation period included pre-ICG injection photographs of all sites, ICG injection, a 5-min video on a selected site using high laser, and post-ICG photographs of all sites for up to 30 min after ICG injection. A 3-photograph sequence was recorded for each site at each time point and included pictures for laser off, laser on low, and laser on high.

Histopathology

Animals were humanely euthanized after the 72-h readings. Full-thickness excisions (including panniculus carnosa) of each entire lesion were performed, and surrounding skin was removed, stapled onto labeled, paraffin-coated index cards, and placed into 10% neutral buffered formalin (NBF). To allow adequate NBF into the tissue, two parallel cuts approximately 1.5 cm apart were made in the skin sections before attachment to index card. Sections were trimmed, paraffin embedded, and stained with hematoxylin and eosin (H&E). A limited number of the histology sections were evaluated by the veterinary pathologist. The remaining slides are waiting evaluation. Evaluation of all sections was conducted in a blinded fashion to determine the depth of the HD lesion.

RESULTS

Histological evaluations were completed for a limited number of experimental sites. A 2-min HD exposure produced a superficial lesion. Histological evaluation demonstrated minimal focal necrosis of epidermis, minimal multifocal areas of hemorrhage and necrosis in superficial dermis (Fig 1a). LDPI and ICGFI showed increased blood flow for the 2-min HD lesion at all observation times (Fig 2). An 8-min HD exposure produced a lesion of intermediate depth. Histological evaluation demonstrated severe diffuse necrosis of epidermis, severe diffuse necrosis of superficial dermis extending to the fat layer, with mild hemorrhage, and no edema (Fig 1b). LDPI and ICGFI showed increased blood flow for the 8-min HD lesion at all observation times (Fig 3). A 30-min HD exposure produced a deep dermal full-thickness lesion. Histological evaluation demonstrated severe diffuse necrosis of epidermis and dermis with severe hemorrhage and edema extending throughout the deep dermis (Fig 1c). LDPI and ICGFI showed decreased blood flow for the 30-min HD lesion at all observation times (Fig 4). A 60-min HD exposure produced a deep full-thickness lesion. Histological evaluation demonstrated severe, diffuse necrosis of epidermis and severe diffuse necrosis, hemorrhage and edema throughout the dermis extending to the deep muscle layer (Fig 1d). LDPI and ICGFI showed decreased blood flow for the 60-min HD lesion at all observation times (Fig 5). Lesion depths were confirmed by histological evaluation on selected samples from each exposure time. A histological evaluation will be completed for all experimental sites.

A summary of the LDPI data is given in Figure 6. This graph gives the mean (+/- SD) blood perfusion ratio comparing lesion skin to normal perilesional skin for HD exposures of 2, 8, 30, and 60 minutes at 24, 48, and 72 hours post-exposure.

A summary of the ICGFI data recorded within 30 seconds of ICG injection is given in Figure 7. This graph gives the mean fluorescence brightness ratio comparing lesion skin with

normal perilesional skin for HD exposures of 2, 8, 30, and 60 minutes at 24, 48, and 72 hours post-exposure. Not enough sites were available to include meaningful error bars.

A summary of the ICGFI data recorded 10 to 30 minutes after ICG injection is given in Figure 8. This graph gives the mean (+/- SD) fluorescence brightness ratio comparing lesion skin with normal perilesional skin for HD exposures of 2, 8, 30, and 60 minutes at 24, 48, and 72 hours post-exposure.

Analysis of the the ICGFI recorded on the video clip shortly after ICG injection (Figure 7) indicated the results reported above and displayed in Figures 2-5. Image analyses on the still photographs taken 10-30 min after ICG injection are summarized in Figure 8. This data set gives no difference in fluorescence between the normal perilesional skin and any of the 2- to 60-min HD exposed sites at any of the observation times. This is contrary to the data from the video clips recorded within 30 sec of ICG injection.

DISCUSSION

Preliminary results demonstrated that LDPI and ICGFI were useful tools to characterize skin perfusion and provided a good estimate of HD lesion depth. The RC data are still being analyzed; however, the initial interpretation suggests that the data do not provide useful information concerning lesion depth.

The LDPI data generated in this study correlate well with previous studies (see Introduction). There have been relatively few reported experiments using the ICGFI technique to evaluate burn depth.^{21,23,24,27} In previous studies, fluorescence was recorded from 5 to 10 min after ICG injection. In our studies, using a new prototype instrument, the fluorescence images recorded shortly after ICG injection correlated best for the degree of lesion depth. The difference in the experimental results may be a function of laser power, ICG injection concentration, or recording distance of the camera. These variables will be evaluated in future studies.

LDPI and ICGFI accurately predicted the need for aggressive treatment (30-and 60-min HD lesions) and non-aggressive treatment (2- and 8-min HD lesions) for the lesions generated in this study.

The LDPI technique is an excellent tool to help the burn surgeon evaluate the lesion depth. It is difficult, however, to apply this technique in a practical way in a normal hospital setting. The LDPI instrument used in this study is large and difficult to position. The size of newer models, however, is somewhat less bulky. The important point is that patients must be positioned under the instrument and remain perfectly still for several minutes during the collection period. In addition, each collection is only for a relatively small skin area depending of the resolution selected.

The novel prototype ICGFI instrument used in this study offered several advantages over LDPI and other ICGFI instruments currently available, including real-time imaging of blood flow through lesions, cost, small size, portability, and not requiring the patient to be re-positioned. A burn surgeon using this prototype instrument could evaluate the entire skin surface in a very short time with only a few injections of ICG. It may even be possible using a CCD

video camera to record the skin fluorescence and re-construct a digital map of the skin indicating what areas need aggressive treatment and what areas are likely to heal on their own.

Proof-of-concept was demonstrated for the prototype ICGFI instrument used in this study. Several additional experiments are still needed to completely define the operational use of this technique. Experiments are required to determine the exposure time necessary to produce a graded series of partial thickness HD lesions. It is important to determine how well this technique will predict the need for aggressive treatment of the truly difficult borderline partial/full thickness lesion. Instrument parameters must be optimized for laser strength, standardized viewing distance from patient, and quality of CCD camera. The optimum quantity of ICG used with each injection must be determined along with establishing the best viewing time post injection. Finally, sufficient numbers of animals must be used to establish statistically significant data to accurately define the optimized procedure to be used.

The data generated in this and the follow-on study will demonstrate the full potential LDPI and ICGFI hold for predicting the need for aggressive treatment following HD exposure.

CONCLUSIONS

1. Proof-of-concept demonstrated for prototype ICGFI instrument.
2. LDPI and ICGFI are useful tools to characterize skin perfusion and provided a good estimate of HD lesion depth.
3. Reflectance colorimetry did not provide an estimate of lesion depth.
4. A 2-min HD liquid exposure produced a superficial lesion. LDPI and ICGFI showed increased blood flow at all observation times and provided a good estimate of lesion depth. Aggressive lesion treatment was not indicated.
5. An 8-min HD liquid exposure produced a lesion of intermediate depth with severe diffuse necrosis of the superficial dermis. LDPI and ICGFI showed increased blood flow at all observation times and provided a good estimate of lesion depth. Aggressive lesion treatment was not indicated.
6. A 30-min HD exposure produced a deep full-thickness lesion with severe diffuse necrosis of epidermis and dermis and severe hemorrhage and edema extending throughout the deep dermis. LDPI and ICGFI showed decreased blood flow at all observation times and provided a good estimate of lesion depth. Aggressive lesion treatment was strongly indicated.
7. A 60-min HD exposure produced a deep full-thickness lesion with severe diffuse necrosis of epidermis and severe diffuse necrosis, hemorrhage and edema throughout the dermis extending to the deep muscle layer. LDPI and ICGFI showed decreased blood flow at all observation times and provided a good estimate of lesion depth. Aggressive lesion treatment was strongly indicated.

8. ICGFI has several advantages over LDPI, including rapid real-time viewing of blood perfusion, small instrument size and portability, low cost, and not requiring the patient to be re-positioned because the instrument moves instead.

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FIGURE CAPTIONS

1.
 - a. Section of skin from the ventral area of weanling swine exposed to 400 μ l of neat sulfur mustard for 2 minutes (H & E). Observed minimal focal necrosis of epidermis, minimal multifocal areas of hemorrhage and necrosis in superficial dermis.
 - b. Section of skin from the ventral area of weanling swine exposed to 400 μ l of neat sulfur mustard for 8 minutes (H & E). Observed severe diffuse necrosis of epidermis, severe diffuse necrosis of superficial dermis extending to the fat layer, with mild hemorrhage, and no edema.
 - c. Section of skin from the ventral area of weanling swine exposed to 400 μ l of neat sulfur mustard for 30 minutes (H & E). Observed severe diffuse necrosis of epidermis and dermis with severe hemorrhage and edema extending throughout the deep dermis.
 - d. Section of skin from the ventral area of weanling swine exposed to 400 μ l of neat sulfur mustard for 60 minutes (H & E). Observed severe diffuse necrosis of epidermis and severe diffuse necrosis, hemorrhage and edema throughout the dermis extending to the deep muscle layer.
2. Composite of representative pictures of lesions on weanling swine exposed to 400 μ l of neat sulfur mustard for 2 minutes. Pictures include normal color photograph, LDPI, and ICGFI at 24, 48, and 72 hours post-exposure.
3. Composite of representative pictures of lesions on weanling swine exposed to 400 μ l of neat sulfur mustard for 8 minutes. Pictures include normal color photograph, LDPI, and ICGFI at 24, 48, and 72 hours post-exposure.
4. Composite of representative pictures of lesions on weanling swine exposed to 400 μ l of neat sulfur mustard for 30 minutes. Pictures include normal color photograph, LDPI, and ICGFI at 24, 48, and 72 hours post-exposure.
5. Composite of representative pictures of lesions on weanling swine exposed to 400 μ l of neat sulfur mustard for 60 minutes. Pictures include normal color photograph, LDPI, and ICGFI at 24, 48, and 72 hours post-exposure.
6. Graph of image analysis giving the mean (+/- SD) LDPI blood perfusion ratio comparing lesion skin with normal perilesional skin for weanling swine exposed to 400 μ l of neat sulfur mustard for 2, 8, 30, and 60 minutes at 24, 48, and 72 hours post-exposure.
7. Graph of image analysis giving the mean ICG fluorescence brightness ratio comparing lesion skin with normal perilesional skin for weanling swine exposed to 400 μ l of neat sulfur mustard for 2, 8, 30, and 60 minutes at 24, 48, and 72 hours post-exposure. Pictures were taken within 30 seconds of ICG injection.

8. Graph of image analysis giving the mean (+/- SD) ICG fluorescence brightness ratio comparing lesion skin with normal perilesional skin for weanling swine exposed to 400 μ l of neat sulfur mustard for 2, 8, 30, and 60 minutes at 24, 48, and 72 hours post-exposure. Pictures were taken 10 to 30 minutes after ICG injection.

Figure 1a. Section of skin from the ventral area of weanling swine exposed to 400 μ l of neat sulfur mustard for 2 minutes (H & E). Observed minimal focal necrosis of epidermis, minimal multifocal areas of hemorrhage and necrosis in superficial dermis.

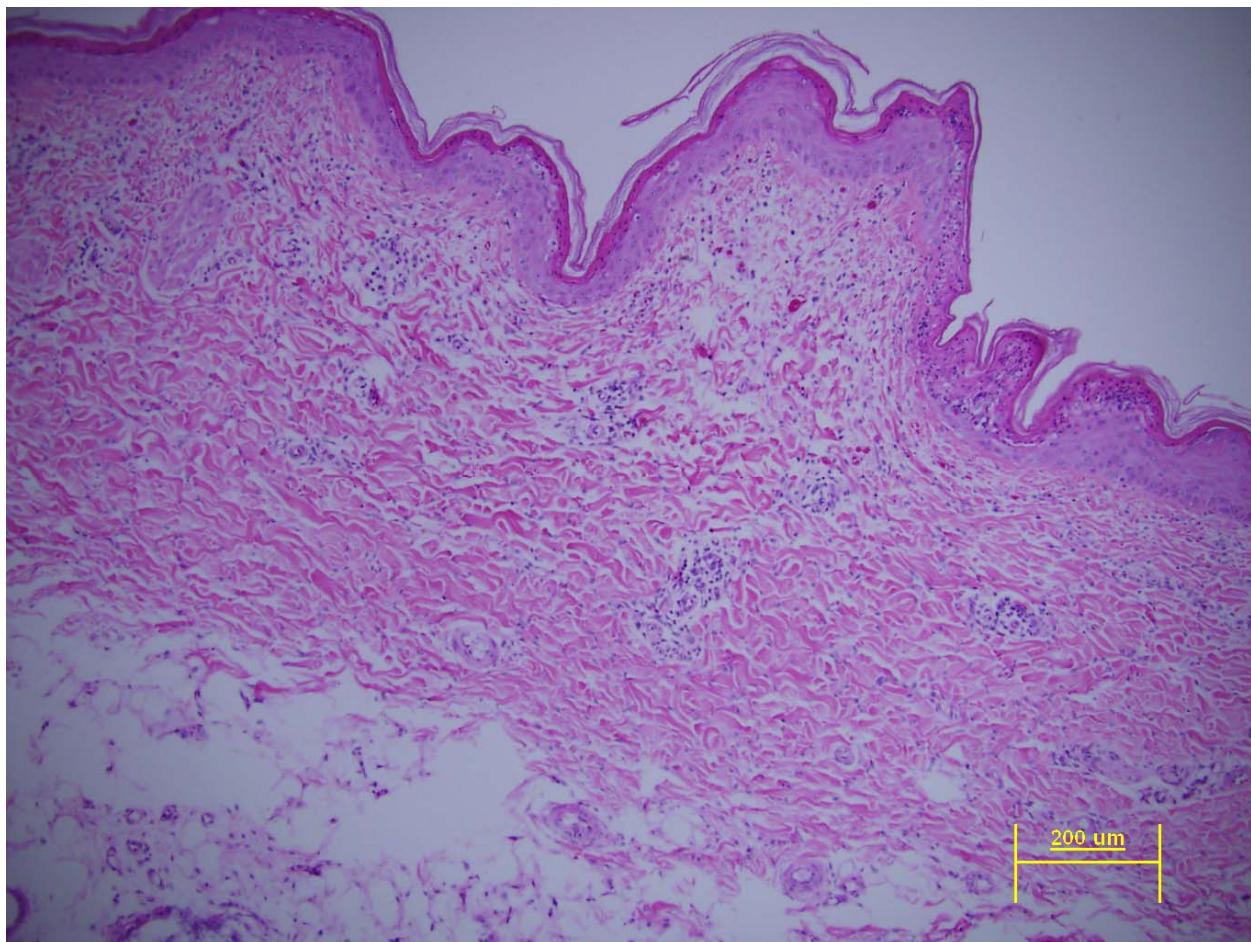


Figure 1b. Section of skin from the ventral area of weanling swine exposed to 400 μ l of neat sulfur mustard for 8 minutes (H & E). Observed severe diffuse necrosis of epidermis, severe diffuse necrosis of superficial dermis extending to the fat layer, with mild hemorrhage, and no edema.

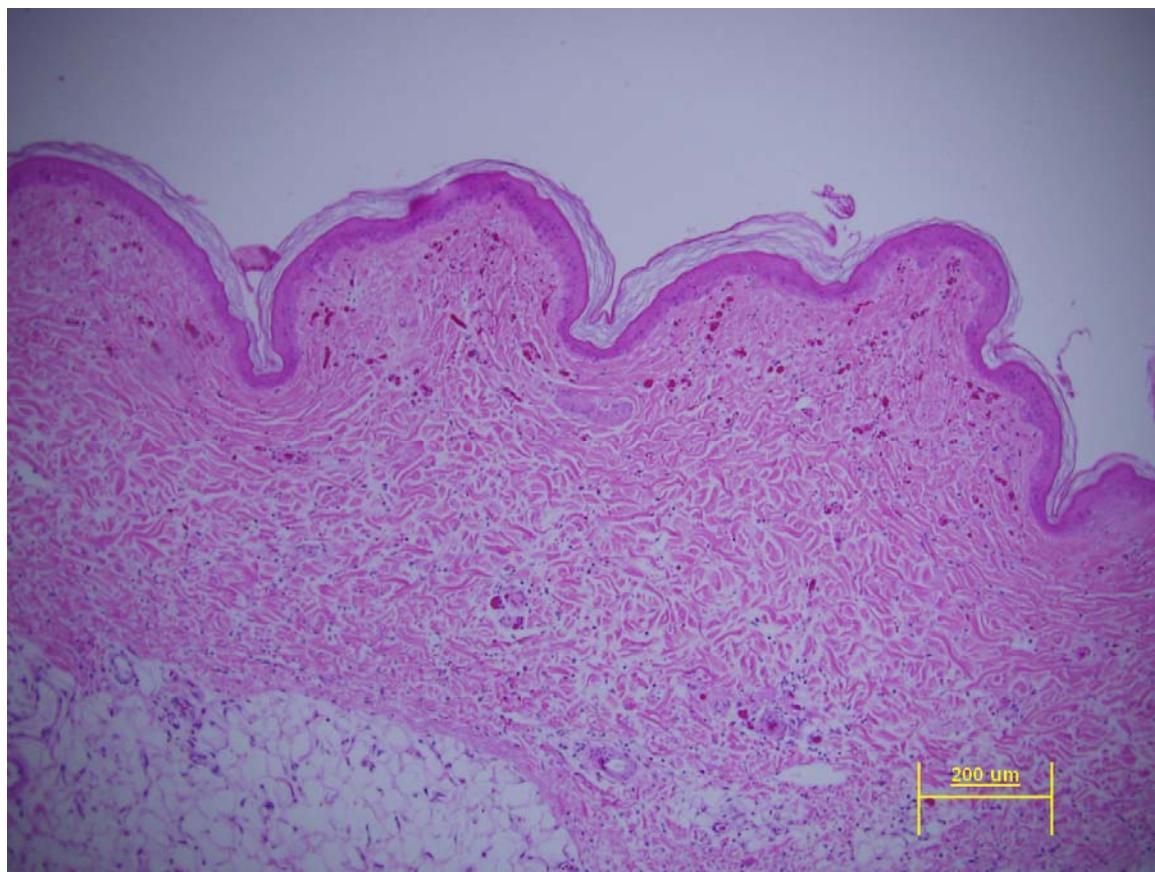


Figure 1c. Section of skin from the ventral area of weanling swine exposed to 400 μ l of neat sulfur mustard for 30 minutes (H & E). Observed severe diffuse necrosis of epidermis and dermis with severe hemorrhage and edema extending throughout the deep dermis.

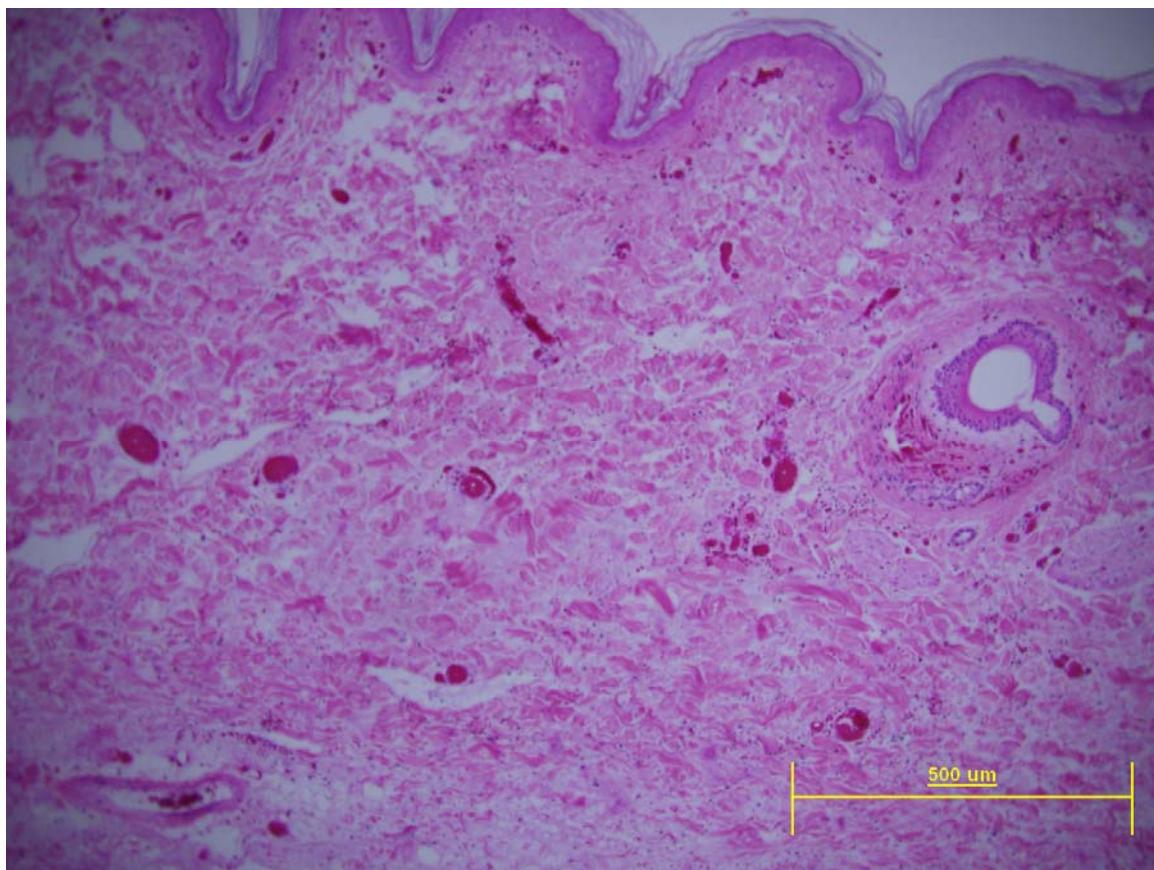


Figure 1d. Section of skin from the ventral area of weanling swine exposed to 400 μ l of neat sulfur mustard for 60 minutes (H & E). Observed severe diffuse necrosis of epidermis and severe diffuse necrosis, hemorrhage and edema throughout the dermis extending to the deep muscle layer.

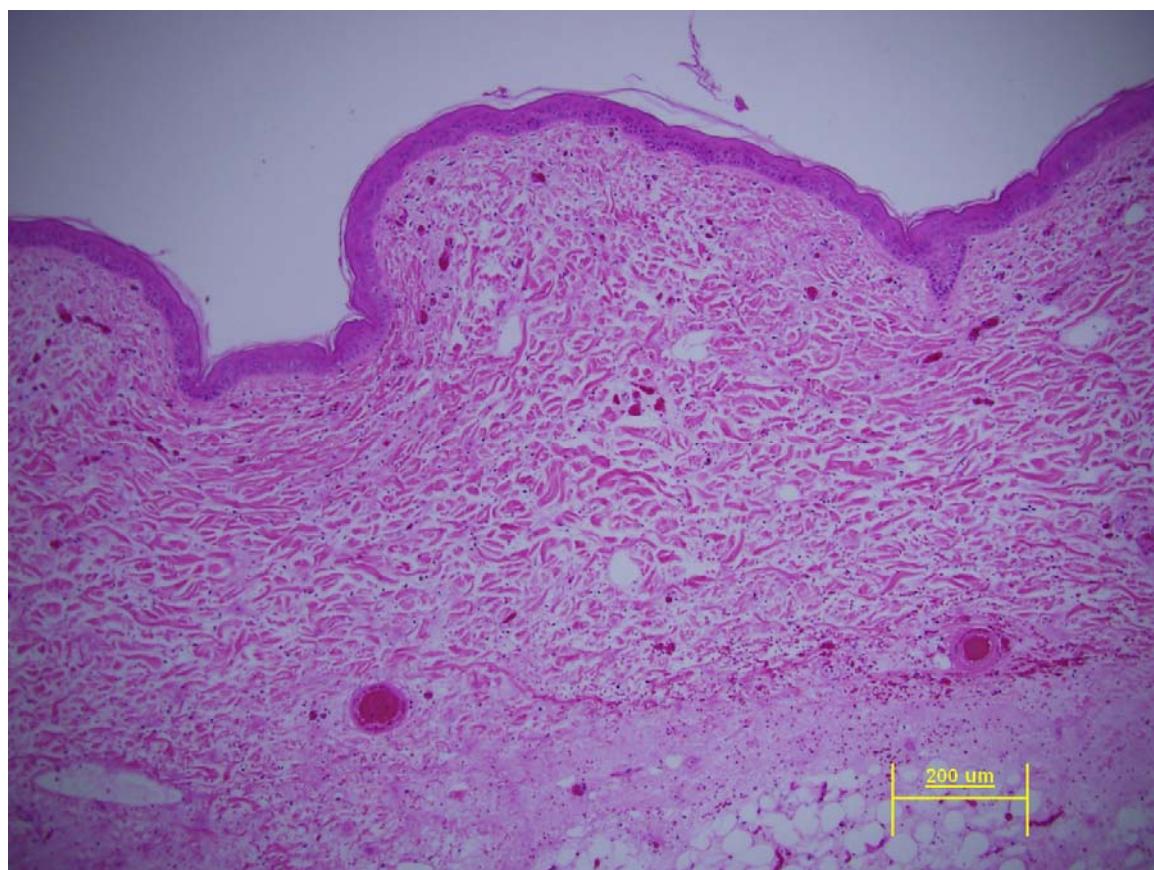


Figure 2. Composite of representative pictures of lesions on weanling swine exposed to 400 μ l of neat sulfur mustard for 2 minutes. Pictures include normal color photograph, LDPI, and ICGFI at 24, 48, and 72 hours post-exposure.

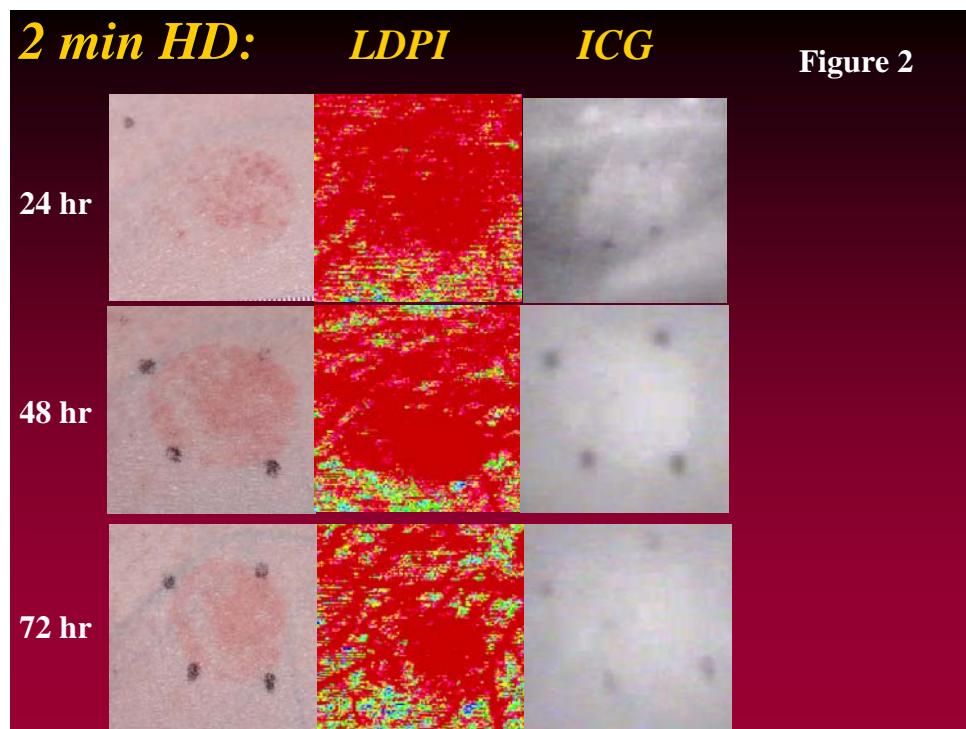


Figure 3. Composite of representative pictures of lesions on weanling swine exposed to 400 μ l of neat sulfur mustard for 8 minutes. Pictures include normal color photograph, LDPI, and ICGFI at 24, 48, and 72 hours post-exposure.

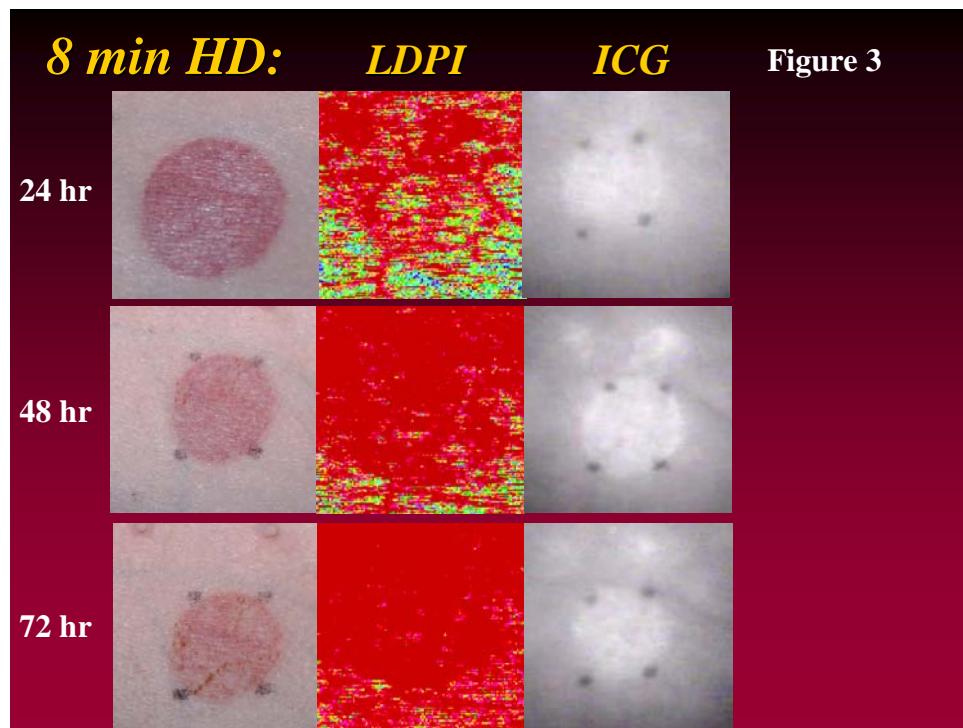


Figure 4. Composite of representative pictures of lesions on weanling swine exposed to 400 μ l of neat sulfur mustard for 30 minutes. Pictures include normal color photograph, LDPI, and ICGFI at 24, 48, and 72 hours post-exposure.

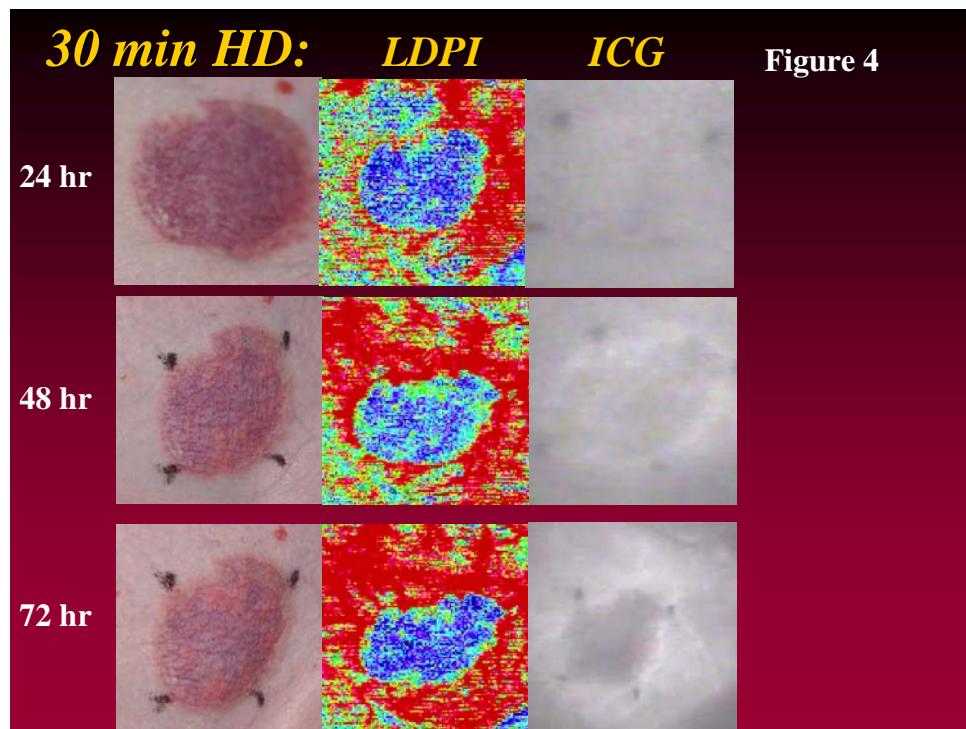


Figure 5. Composite of representative pictures of lesions on weanling swine exposed to 400 μ l of neat sulfur mustard for 60 minutes. Pictures include normal color photograph, LDPI, and ICGFI at 24, 48, and 72 hours post-exposure.

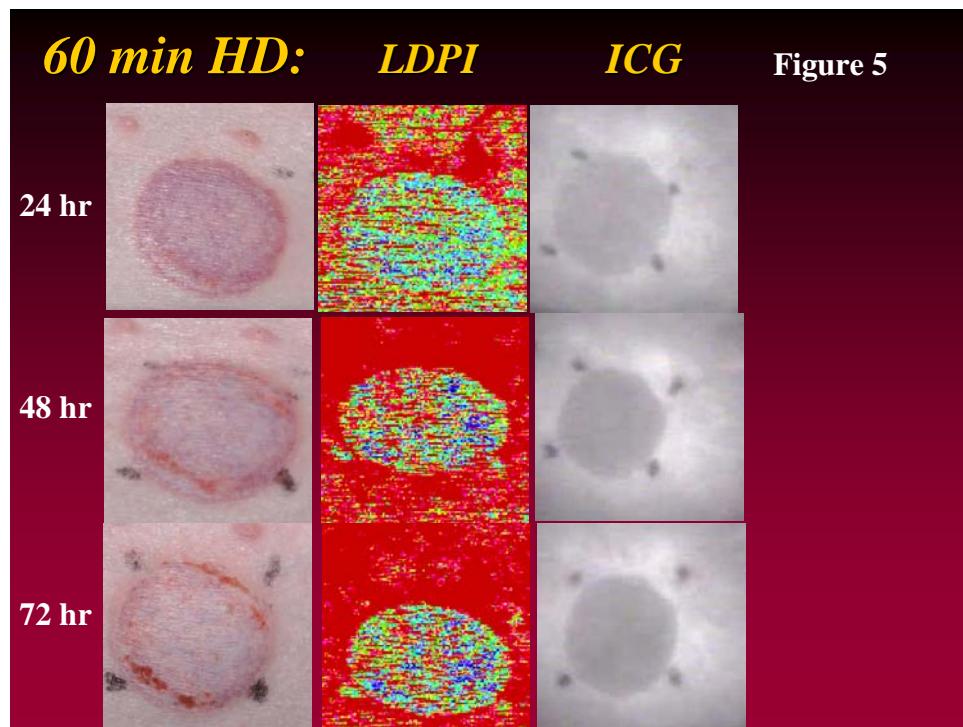


Figure 6. Graph of image analysis giving the mean (+/- SD) LDPI blood perfusion ratio comparing lesion skin with normal perilesional skin for weanling swine exposed to 400 μ l of neat sulfur mustard for 2, 8, 30, and 60 minutes at 24, 48, and 72 hours post-exposure.

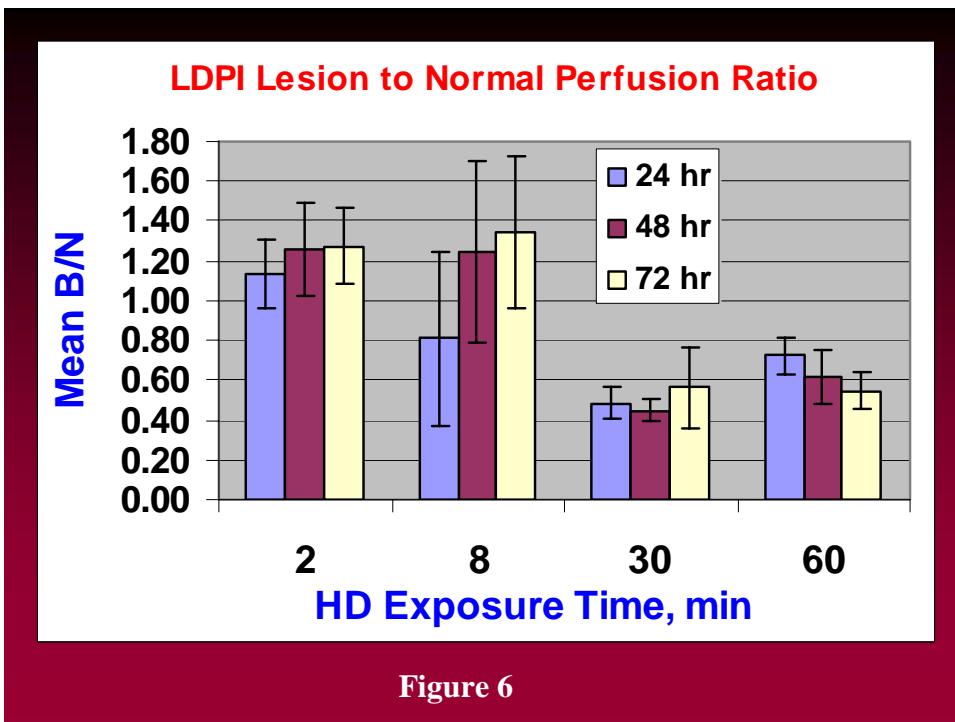


Figure 7. Graph of image analysis giving the mean ICG fluorescence brightness ratio comparing lesion skin with normal perilesional skin for weanling swine exposed to 400 μ l of neat sulfur mustard for 2, 8, 30, and 60 minutes at 24, 48, and 72 hours post-exposure. Pictures were taken within 30 seconds of ICG injection.

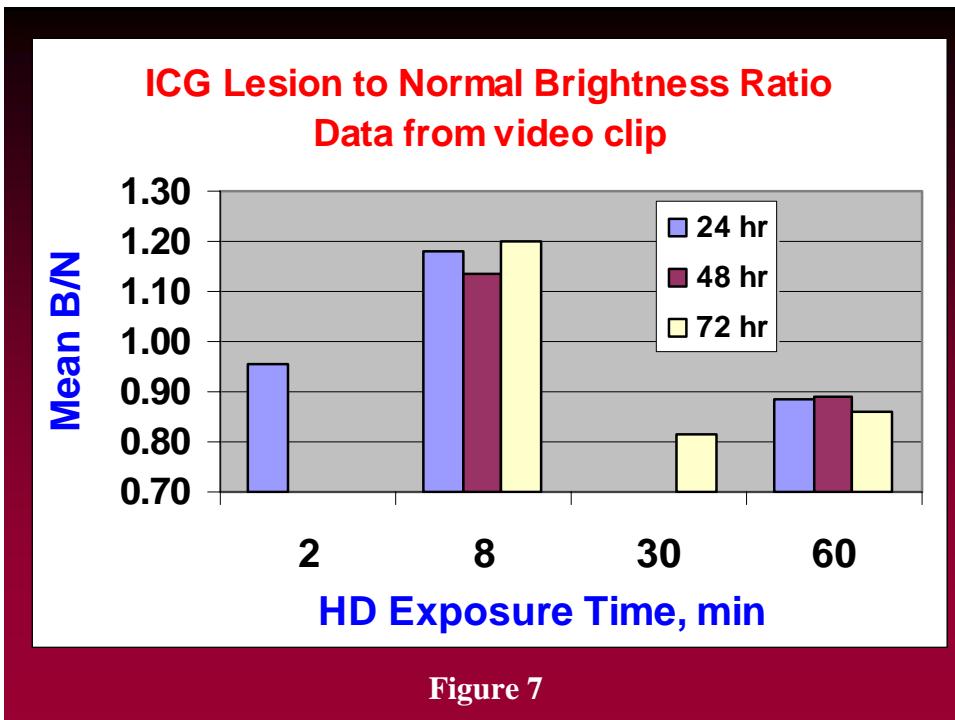


Figure 8. Graph of image analysis giving the mean (+/- SD) ICG fluorescence brightness ratio comparing lesion skin with normal perilesional skin for weanling swine exposed to 400 μ l of neat sulfur mustard for 2, 8, 30, and 60 minutes at 24, 48, and 72 hours post-exposure. Pictures were taken 10 to 30 minutes after ICG injection.

